REMARKS

By the present invention, there are provided recombination systems, DNA constructs, cells, and nonhuman mammals useful for the site-specific integration and excision of nucleic acids into and out of the genomes of host cells.

The novel site-specific recombination system of the present invention provides artisans with the ability to target the integration of transfected DNA to a specific chromosomal site in mammalian host cells at frequencies exceeding that of both random and other site-specific integration systems. Additionally, the invention recombination system allows for immediate confirmation and analysis of the recombination event. Applicants' recombination system is distinctive in its precision and predictability, providing methods which enable artisans to routinely create or disrupt a functional translational reading frame at an intended site of integration.

By the present communication, claims 2-6, 8-12, 14-18, 29-30, 32-36 and 39-41 have been amended to define Applicants' invention with greater particularity. No new matter is introduced by the subject amendments as all amended claim language is fully supported by the specification and original claims.

Accordingly, claims 1-18 and 29-41 are currently under examination.

Applicants respectfully traverse the rejection of claims 29-34 under 35 U.S.C. §101, as allegedly claiming nonstatutory subject matter. Claim 29 (from which claims 30-34 depend) has been amended herewith so as to require that the claimed mammalian cell is either a nonhuman cell or an isolated human cell. In view of this amendment, it is respectfully submitted that this rejection has been rendered moot. Accordingly, withdrawal of this rejection is respectfully requested.

Applicants respectfully traverse the rejection of claims 1-18 and 29-41 under 35 U.S.C. §112, first paragraph, as allegedly failing to provide a reasonable written description and enablement for practicing the claimed invention.

Contrary to the Examiner's concerns, the integration of the first FRT site into the genome is not required by the claims to be a targeted event. It is only the recombination of the second FRT with the first FRT that is required to be precisely targeted (i.e., this recombination event must occur precisely at the location of the first FRT in the genome, wherever that may be; See the specification at p. 12, lines 28-32).

As explained in the specification, the introduction of DNA encoding the first FRT is "accomplished employing standard techniques" (See the specification at p. 15, lines 4-9). These standard techniques do not allow the precise targeting of the first FRT, as only techniques that allow for random insertion of exogenous DNA have been described in the art (See the specification at p. 2, lines 10-14). Thus, it is not necessary for Applicants to provide a specific written description of means for integrating the first FRT into the genome, as this information is already present in the art, and is therefore considered as much a part of Applicants' specification as if it had been incorporated fully.

Applicants respectfully traverse the rejection of claims 1-18 and 29-41 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. In view of the claim amendments submitted herewith, Applicants respectfully submit that these rejections have been rendered moot. Thus, for example, the claims have been amended to remove the allegedly indefinite term "portion", which has been replaced with the term --segment--; claims 2, 30 and 36 have been amended to provide antecedent basis for the phrase "said first gene of interest"; the term "derived" has been removed from claims 8-9 and 15-17; claims 10 and 18 have been amended to delete the phrase "approximately 1450 base pair", and to include reference to --SEQ ID NO:1--; claims 29 and 35 have

been amended to include reference to "said first DNA"; claims 34 and 41 have been amended to provide antecedent basis for the phrase "said second DNA"; claims 11, 12, 32 and 39 have been amended to remove reference to "the host system" (which lacked antecedent basis); and claims 33 and 40 have been amended to require that the FLP recombination target site have the specific sequence given.

Applicants respectfully disagree with the Examiner's suggestion that the term "FLP" should be spelled out. It is respectfully submitted that this term is typically used in the art in exactly this form, and no confusion is possible to those skilled in the art.

Claims 8-9 and 15-17 have been amended to delete therefrom the term "derived". This amendment makes it clear that Applicants are not claiming FLP recombinases (claims 8-9 and 16-17) or gene segments (claim 15) that are modified from the indicated starting materials. Instead, it is respectfully submitted to be clear that these claims embrace proteins or DNAs that are either isolated directly from the starting materials indicated, or cloned from those starting materials.

Applicants respectfully disagree with the Examiner's assertion that claims 13 and 14 are not clear since the orientation and operability of the recited parts are not specified. As readily understood by those skilled in the art,

the various elements of claims 13 and 14 may exist in any order. Thus, claims 13 and 14 are respectfully submitted to be clear as written.

For all of the above reasons, and in view of the claim amendments submitted herewith, it is respectfully submitted that all of the pending claims fully satisfy the requirements of 35 U.S.C. §112, second paragraph.

Applicants respectfully traverse the rejection of claims 33 and 40 under 35 U.S.C. § 112, fourth paragraph, as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. In view of the amendments to claims 33 and 40 submitted herewith (requiring that the FLP recombination target site have the specific sequence given), it is respectfully submitted that claims 33 and 40, as amended, properly depend from claims 29 and 35, respectively.

Applicants respectfully traverse the rejection of claims 1-18 and 29-34 under 35 U.S.C. §103 over Sauer, U.S. Patent No. 4,959,317, taken with Golic et al., *Cell*, 59:499-509 (1989), Schiestl, U.S. Patent No. 4,997,757, and Senecoff et al., *Proc. Natl. Acad. Sci. USA*, 82:7270-74 (1985). Applicants also respectfully traverse the rejection of claims 35-41 under 35 U.S.C. §103 over Sauer taken with Golic, Schiestl and Senecoff, as applied to claims 1-18 and 29-34, and further in view of Palmiter et al., *Ann. Rev. Genet.*, 20:465-499 (1986).

Applicants' invention, as defined by all of the claims, distinguishes over the art by requiring a mammalian recombination system, as well as methods for promoting recombinations between FRTs in the genome. See O'Gorman et al., *Science*, 251:1351-54 (1991), at p. 1354, column 1, second paragraph, and the paragraph bridging columns 1 and 2. In contrast, Golic discusses recombination between FRTs in *Drosophila melanogaster*, i.e., in insect cells. Although Golic does make the statement that "we expect that [the FLP recombinase system] will work in other organisms as well", this is mere speculation, and does not demonstrate that those skilled in the art enjoyed a reasonable expectation of success if they attempted to utilize the FLP recombinase system in other organisms unrelated to *Drosophila*.

The recombination system taught by Sauer utilizes a protein from bacteriophage P1, *Cre*, and its recombination target, *lox*, to effectuate recombination and integration of DNA. The Sauer recombination system is disclosed to be effective for incorporation of DNA into the chromosomes of non-mammalian species (the yeast *S. cerevisiae*), or into target sites on extrachromosomal plasmids of mammalian cells (mouse). However, Sauer does not disclose or suggest recombination systems useful for genomic modification of mammalian cells. Moreover, the teachings of Sauer are not related to the FLP recombinase system, and do not create a reasonable expectation of success for the utilization of the FLP recombinase system in mammals.

Further reliance upon Schiestl is unable to cure the deficiencies of the primary references, as Schiestl deals with systems for determining the carcinogenicity of test chemicals. This is done by quantifying the number of recombination events catalyzed by the test chemicals. This is unrelated to the invention method of inserting or excising DNA from the genome in a site-specific manner. Indeed, the Schiestl system has nothing to do with FLP-mediated recombination. In fact, to the extent that Schiestl mentions FLP recombination, he teaches the inactivation of the FLP/FRT system in yeast strains being used, so that FLP-mediated recombination does not interfere with quantifying the recombinations catalyzed by the carcinogenic test chemical. See, for example, col. 22, lines 46-50.

Further reliance upon Senecoff is unable to cure the deficiencies of the primary references. Senecoff merely discusses the FLP recombination system in further detail, but does not contain any teaching or suggestion that the FLP recombination system would be functional in a mammalian system.

Further reliance on Palmiter, in combination with the above references, is unable to cure the deficiencies thereof. Palmiter does not relate in any way to the FLP/FRT system, let alone its use in mammalian cells to promote genomic recombination. Accordingly, it is respectfully submitted that the Palmiter reference is irrelevant with regard to the pending claims.

In view of the above amendments and remarks, reconsideration and favorable action on all pending claims are respectfully requested. If any minor matters remain to be resolved, the Examiner is invited to contact the undersigned at the telephone number set forth below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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